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April 04, 2005

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APPLICATION NUMBER: *60/527,608*
FILING DATE: *December 05, 2003*

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I hereby certify that this paper or fee is being deposited with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

By: Denise M Kettelberger
Name: Denise M. Kettelberger

REQUEST FOR PROVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(c)

MAIL STOP PROVISIONAL PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is a request for filing a Provisional application for patent under 37 CFR § 1.53(c) entitled MOLECULAR CLASSIFICATION OF TAMOXIFEN-RESISTANT BREAST CARCINOMAS BY GENE EXPRESSION PROFILING by the following inventor(s):

Full Name Of Inventor	Family Name	First Given Name	Second Given Name
Residence & Citizenship	City	State or Foreign Country	Country of Citizenship
Post Office Address	Post Office Address	City	State & Zip Code/Country
Full Name Of Inventor	Family Name	First Given Name	Second Given Name
Residence & Citizenship	City	State or Foreign Country	Country of Citizenship
Post Office Address	Post Office Address	City	State & Zip Code/Country
Full Name Of Inventor	Family Name	First Given Name	Second Given Name
Residence & Citizenship	City	State or Foreign Country	Country of Citizenship
Post Office Address	Post Office Address	City	State & Zip Code/Country

1. ☒ Enclosed is the Provisional application for patent as follows: 3 pages of specification, and 8 sheets of drawings.

2. ☒ Small entity status is claimed pursuant to 37 CFR 1.27.

3. ☒ Payment of Provisional filing fee under 37 C.F.R. § 1.16(k) :
   - ☐ Attached is a check in the amount of $.
   - ☐ Please charge Deposit Account No. 13-2725.
   - ☒ PAYMENT OF THE FILING FEE IS BEING DEFERRED.

4. ☒ The Commissioner is hereby authorized to charge any additional fees as set forth in 37 CFR §§ 1.16 to 1.18 which may be required by this paper or credit any overpayment to Account No. 13-2725.

5. ☐ Enclosed is an Assignment of the invention to , Recordation Form Cover Sheet and a check for $ to cover the Recordation Fee.

6. ☐ Also Enclosed:

7. ☐ The invention was made by the following agency of the United States Government or under a contract with the following agency of the United States Government:

8. ☒ Address all future communications to the **Attention of Denise M. Kettelberger** (may only be completed by attorney or agent of record) at the address below.

9. ☒ A return postcard is enclosed.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, MN 55402-0903
612/332-5300

23552
PATENT TRADEMARK OFFICE

Date: 5 December 2003

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DMK:lek

PREDICTING RESPONSE TO ANTI-ESTROGEN THERAPY

E.M.J.J. Berns

Erasmus MC Daniel den Hoed Cancer Clinic
Rotterdam, the Netherlands

It would be of great benefit to predict response of metastatic tumors to chemotherapeutic agents. For example, analysis of primary tumors removed by surgery may predict response of later developed metastatic tumors to chemotherapeutic agents.

Attempts have been made to correlate patient response to tamoxifen with specific markers, as shown in Figure 1. See, for example, Berns, Klijn, and Foekens, *J. Clin. Oncol; J. Nat. Cancer Res.,* and *Cancer Res.*

Summary

A gene profile has been correlated with estrogen positive breast cancer tumors and patient response to the anti-estrogen, tamoxifen in the treatment of metastatic disease. Using a gene profile according to the invention, analysis of the patient's primary tumor against the gene profile is predictive of patient response to anti-estrogen, for example, tamoxifen, therapy for the treatment of metastatic disease. See Figure 2.

The invention includes a specific gene profile that is predictive of breast cancer response to the anti-estrogen, tamoxifen, articles, kits, and assay systems utilizing a predictive gene profile of the invention, and methods for detecting patient response to anti-estrogen therapy using a gene profile according to the invention.

Examples

As shown in Figure 3, using breast tumor tissue available from the Erasmus MC Daniel den Hoed Cancer Clinic Medical Onclology tissue bank, a retrospective study was performed to analyze RNA produced from estrogen receptor positive primary breast tumors. Samples of RNA were obtained from the breast cancer tissue and analyzed against 18,500 clones and controls on an NKI 18.5K cDNA microarray (NKI/NKB, microarray.nki.nl). RNA samples were analyzed in duplicate by cDNA microarray analysis on an NKI 18.5K cDNA Microarray (See Figure 8).

Retrospective data collected and matched to the tissue samples was evaluated to "train" the data set, and identify those markers that comprise a data set or

gene profile predictive of patient response to tamoxifen. As shown in Figure 4, tissue and samples from 46 patients was used in the training set. Of the 46 patients, 25 were refractory to tamoxifen and developed progressive disease (PD) and 21 showed an objective response to tamoxifen (OR). The assay performance is shown in Figure 5. Two clusters are apparent: Cluster 1 correlating with progressive disease (PD) or lack of response to tamoxifen (red bar profile) and Cluster 2 (green bar profile) showing the profile of objective response.

Eighty-one genes were identified as useful in the predictive gene profile. As shown in Figure 5, many of these are related genes.

A second set of estrogen receptor positive patient tissue and samples was analyzed to validate the gene profiles. The validation set consisted of 58 patient samples. RNA was prepared and analyzed as described above for the training set. Using the gene profile obtained, a prediction was made for the patient's responsiveness to tamoxifen therapy for treatment of metastasis. The predictions and patient correlations are shown in Figure 5.

An alternative method for gene profile analysis is use of Q-PCR. As shown in Figure 6, both methods are satisfactory for use in the invention.

Figure 7 compares the ability to predict progression free-survival using a traditional factors score with the prediction available with the gene profile of the invention (sensitive shown green; resistant shown red)(response to tamoxifen).

The gene profiles determined by the method described above and shown in the figures enables sensitive and specific prediction of patient response or lack of response to anti-estrogen therapy for metastatic estrogen receptor positive breast cancer .

We claim:

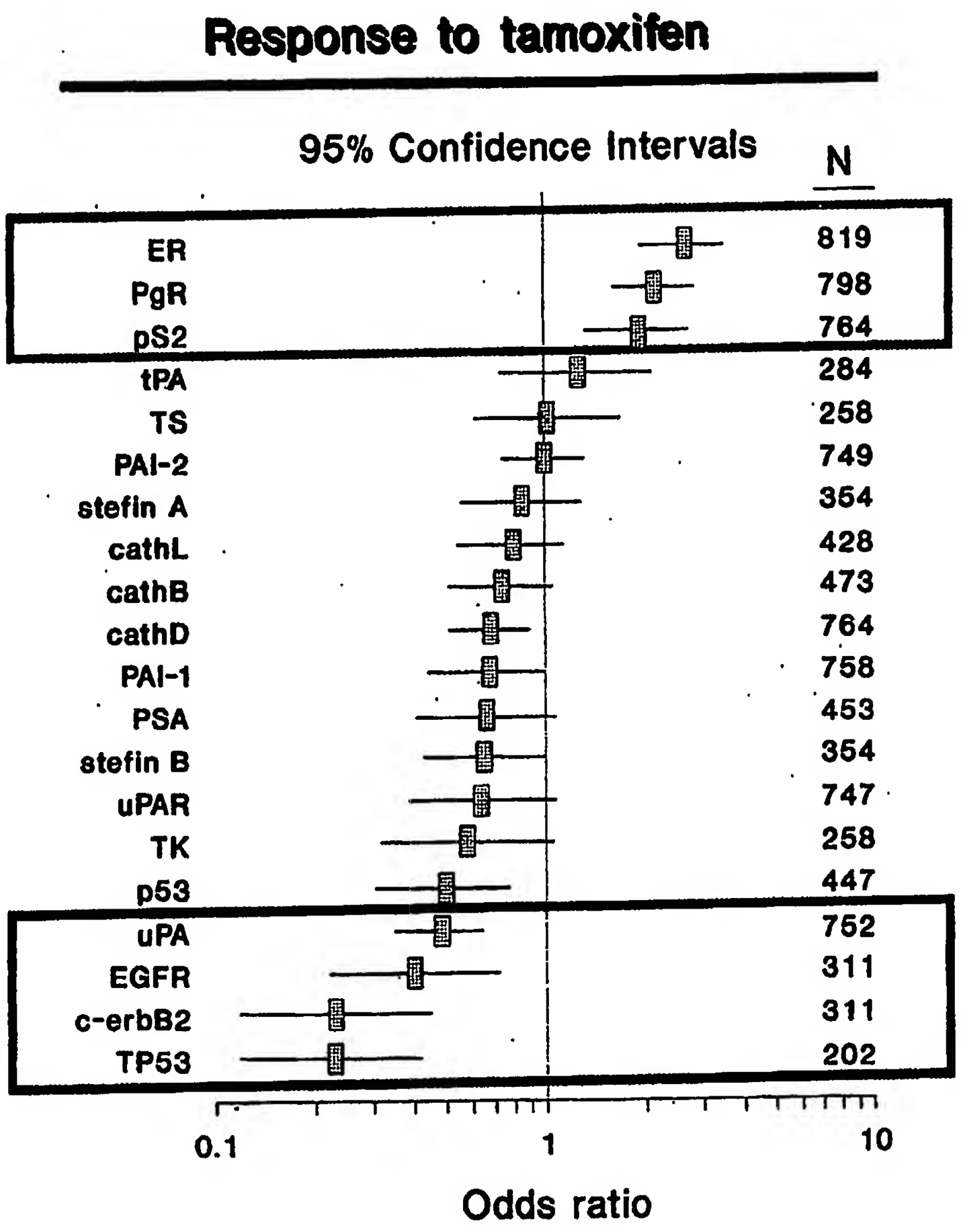
1. A gene profile predictive of estrogen receptor positive breast cancer patient response to anti-estrogen therapy for the treatment of metastatic estrogen receptor positive breast cancer comprising one or more of the profiles shown in Figure 5 or 6.

2. A gene profile predictive of estrogen receptor positive breast cancer patient response to anti-estrogen therapy for the treatment of metastatic estrogen receptor positive breast cancer produced by the method described in the Examples above.

3. A method for predicting patient response to tamoxifen in the treatment of metastatic estrogen receptor positive breast cancer, the method comprising:
analyzing the patient's primary tumor tissue for gene expression; and
correlating a Cluster 2 gene profile as shown in Figure 5 or 6 with predicted response to anti-estrogen therapy.

4. A method for predicting patient response to tamoxifen in the treatment of metastatic estrogen receptor positive breast cancer, the method comprising:
analyzing the patient's primary tumor tissue for gene expression; and
correlating a Cluster 1 gene profile as shown in Figure 5 or 6 with predicted lack of response to anti-estrogen therapy.

Fig.1 Molecular factors 1980-2000



Berns, Klijn & Foekens

J Clin Oncol
J Nat Cancer Inst
Cancer Res arch

Erasmus MC

Fig. 2 Gene expression profiling:

Analysis of the type of response on tamoxifen therapy

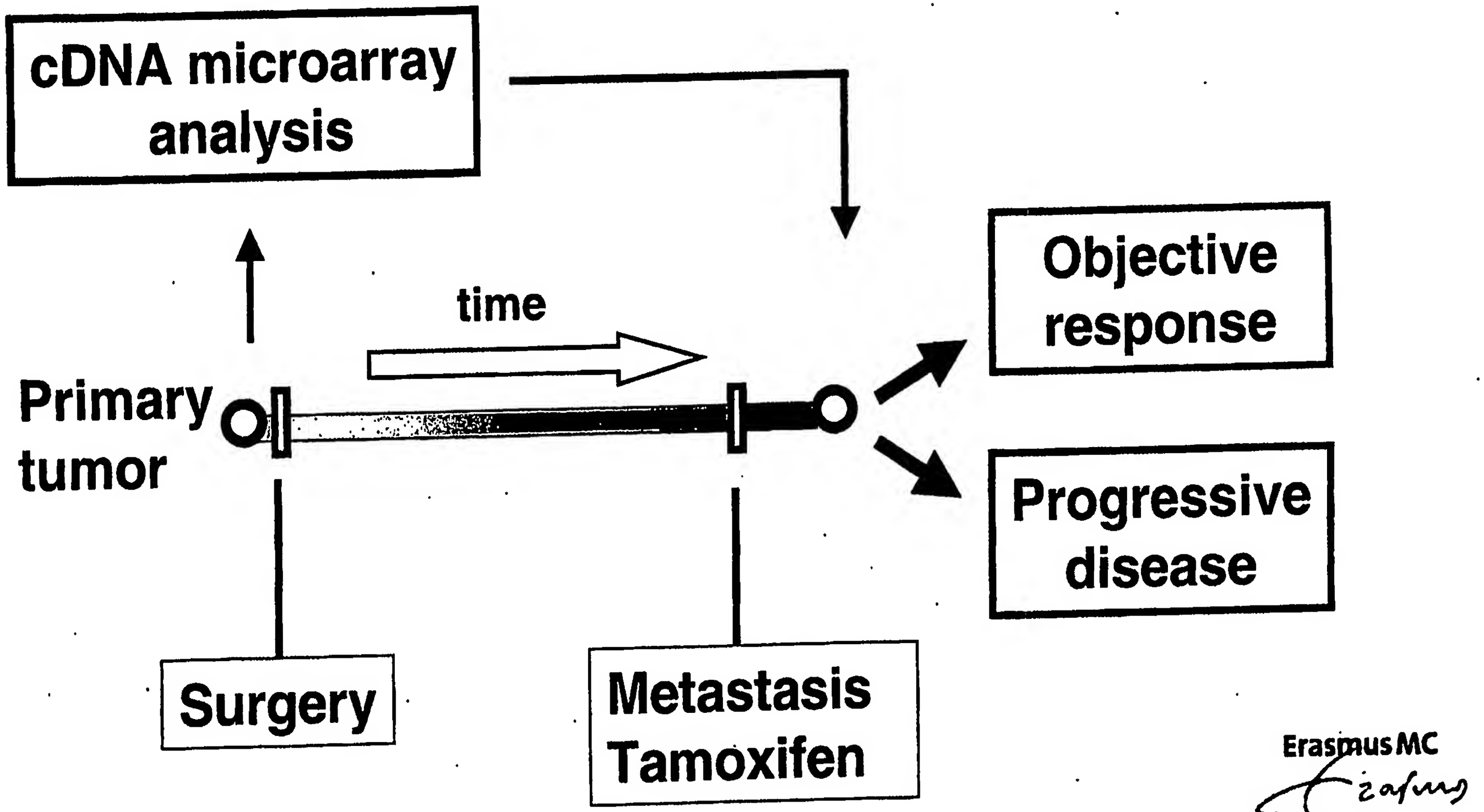


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Fig. 3 Study design

Frozen breast tumors, retrospective

from tissue bank Medical Oncology: with follow-up tamoxifen monotherapy response

RNA aRNA

cRNA: Cy3 and Cy5 (tumor/reference)

18K Human cDNA arrays

18.500 clones (genes, ESTs) & controls non-commercial, NKI/NKB

http://microarrays.nki.nl

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Fig. 4 Data analysis approach – ER+ Only

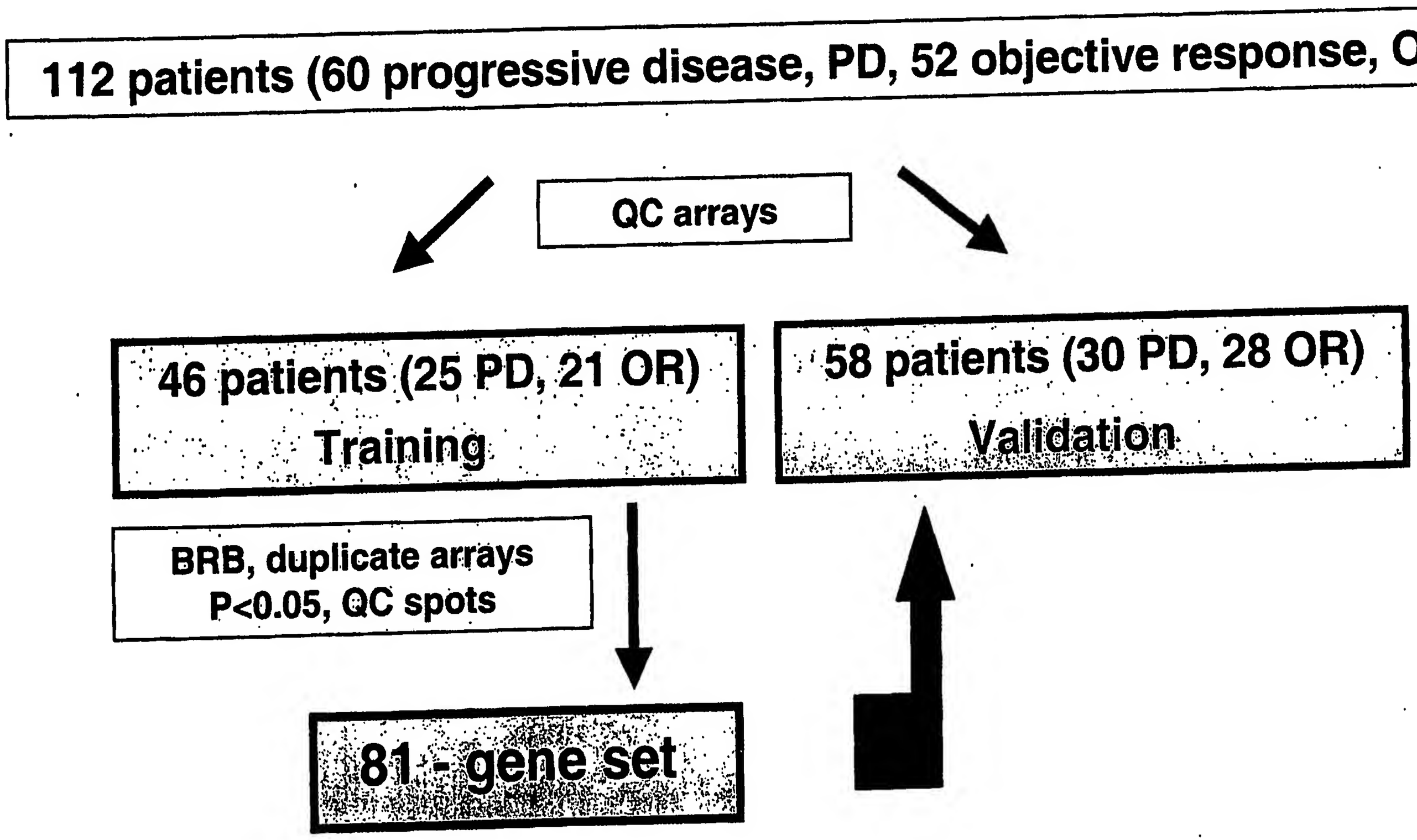

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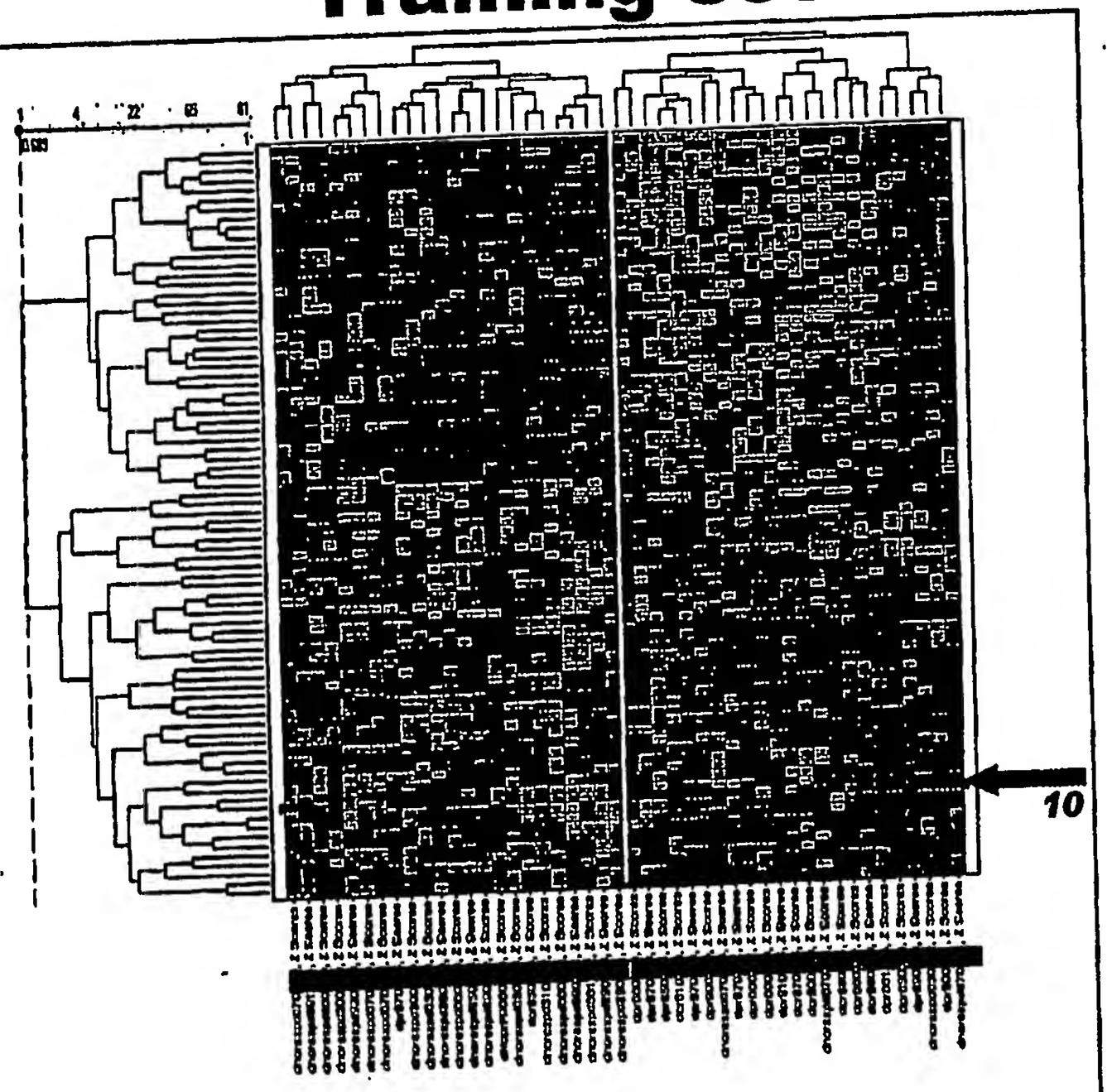
Fig. 5 Assay Performance in ER+ breast tumors: Progressive disease(PD) vs objective response (OR)

Training set



Cluster 1:

PD: 21/23

PPV: 91%

Cluster 2:

OR: 19/23

NPV: 83%

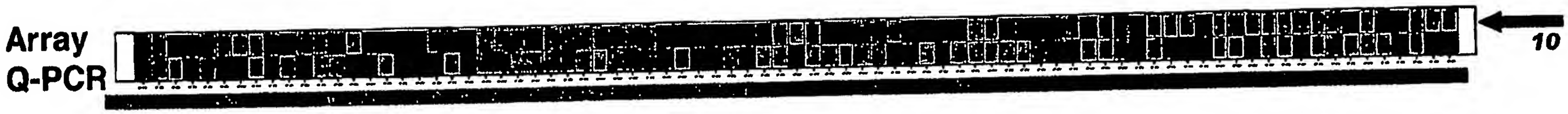
Validation set

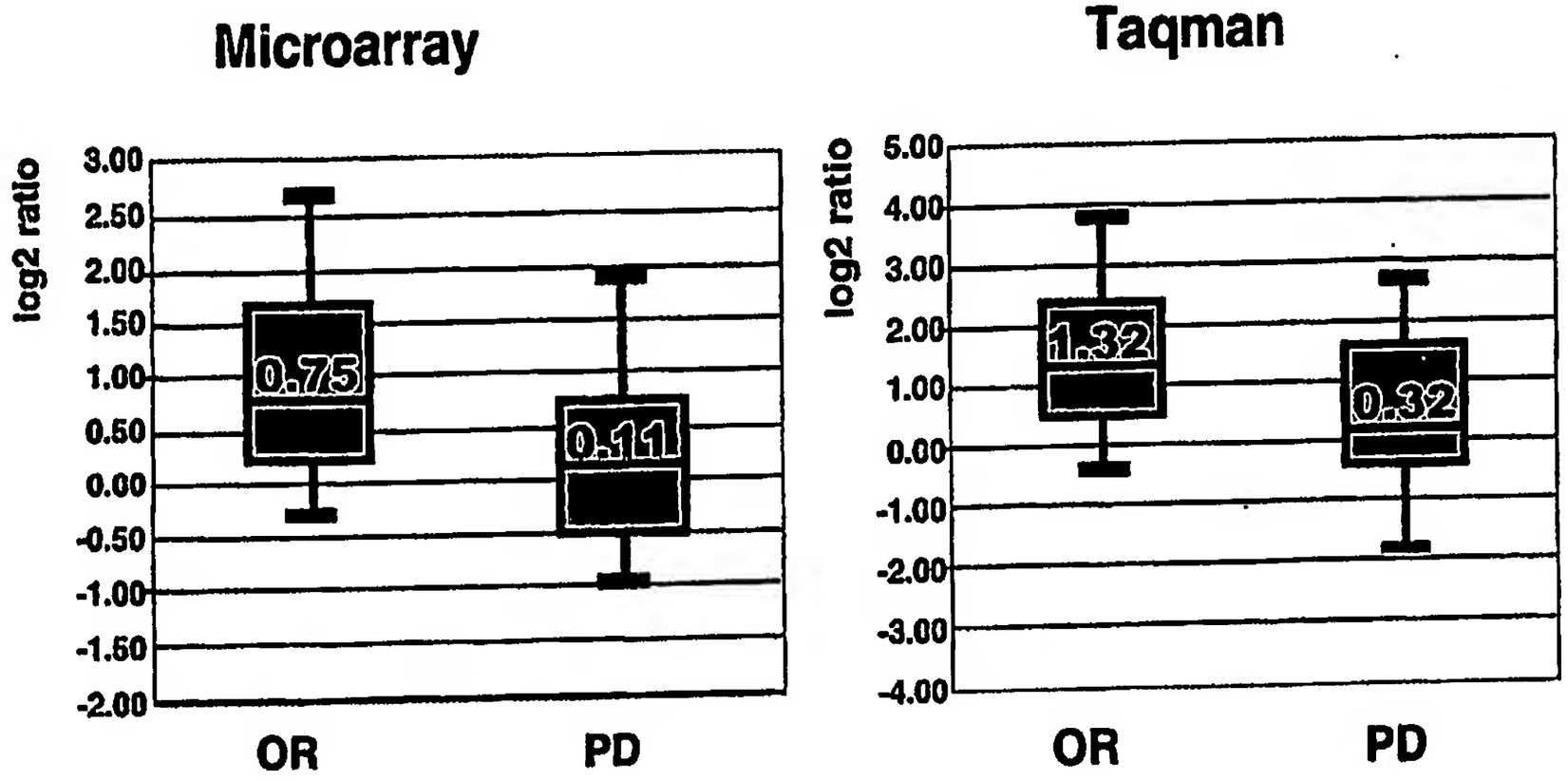
Analysis of 58 ER+ patients

	Actual PD	Actual OR
Predicted		
PD	24	16
OR	6	12

Sensitivity:	80 % (0.67– 0.91)
Specificity:	43 % (0.29 – 0.54)
PPV:	60 %
NPV:	66 %
Odds Ratio:	3 (95% CI: 0.9-9.6)

Fig. 6 Comparison microarray and Q-PCR





Microarray vs Taqman (n=81)

Spearman: R=0.62

(P<0.001)

PD (N=44) *vs* OR (N=37)

Wilcoxon:

Microarray: P=0.0003

Taqman: P=0.007

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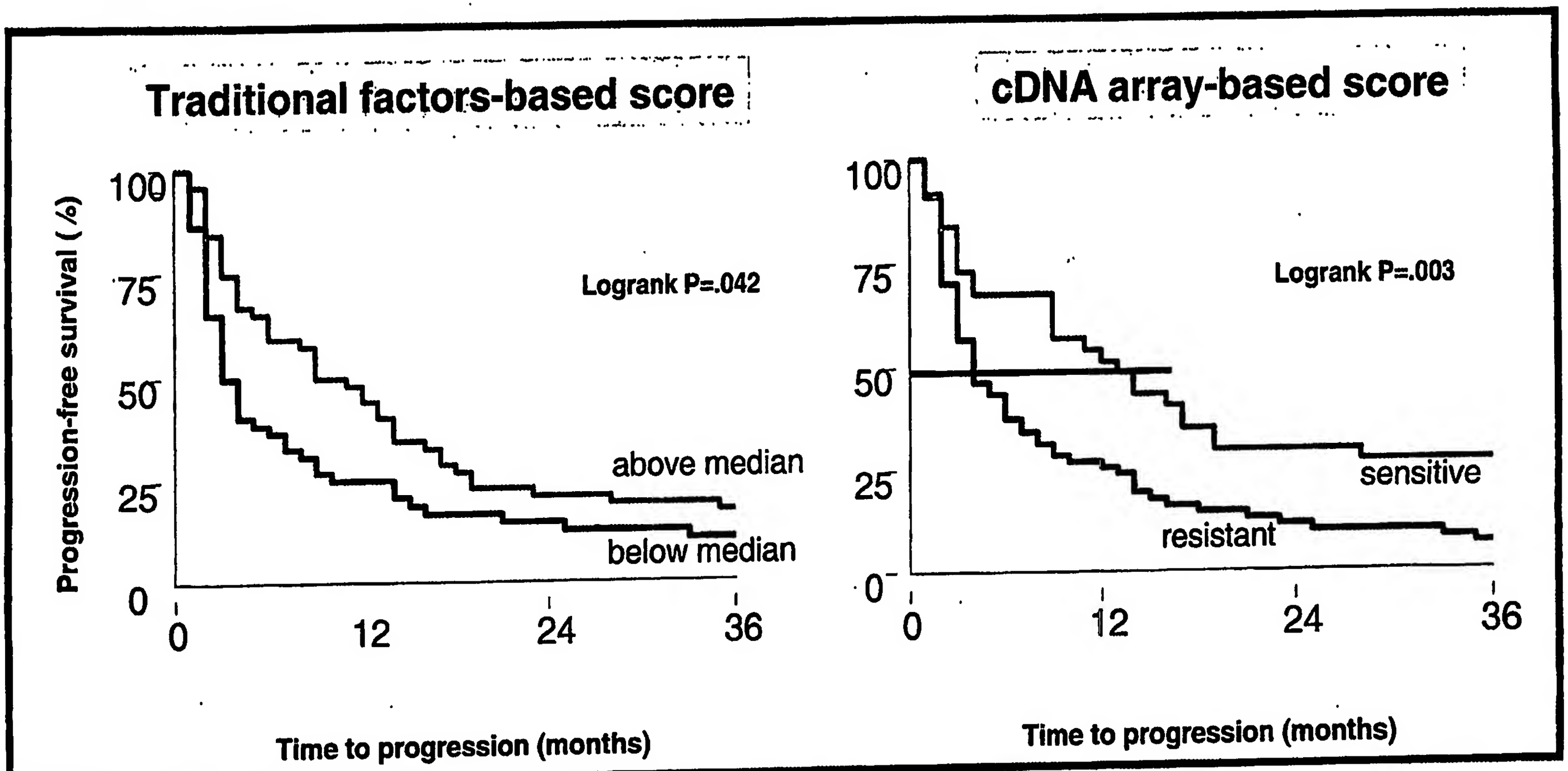
Fig. 7 Prediction of progression-free survival



Tumor & patient characteristics: menopause, disease-free interval, ite of relapse & ER level (log[ER])

Predictive profile of 81 genes

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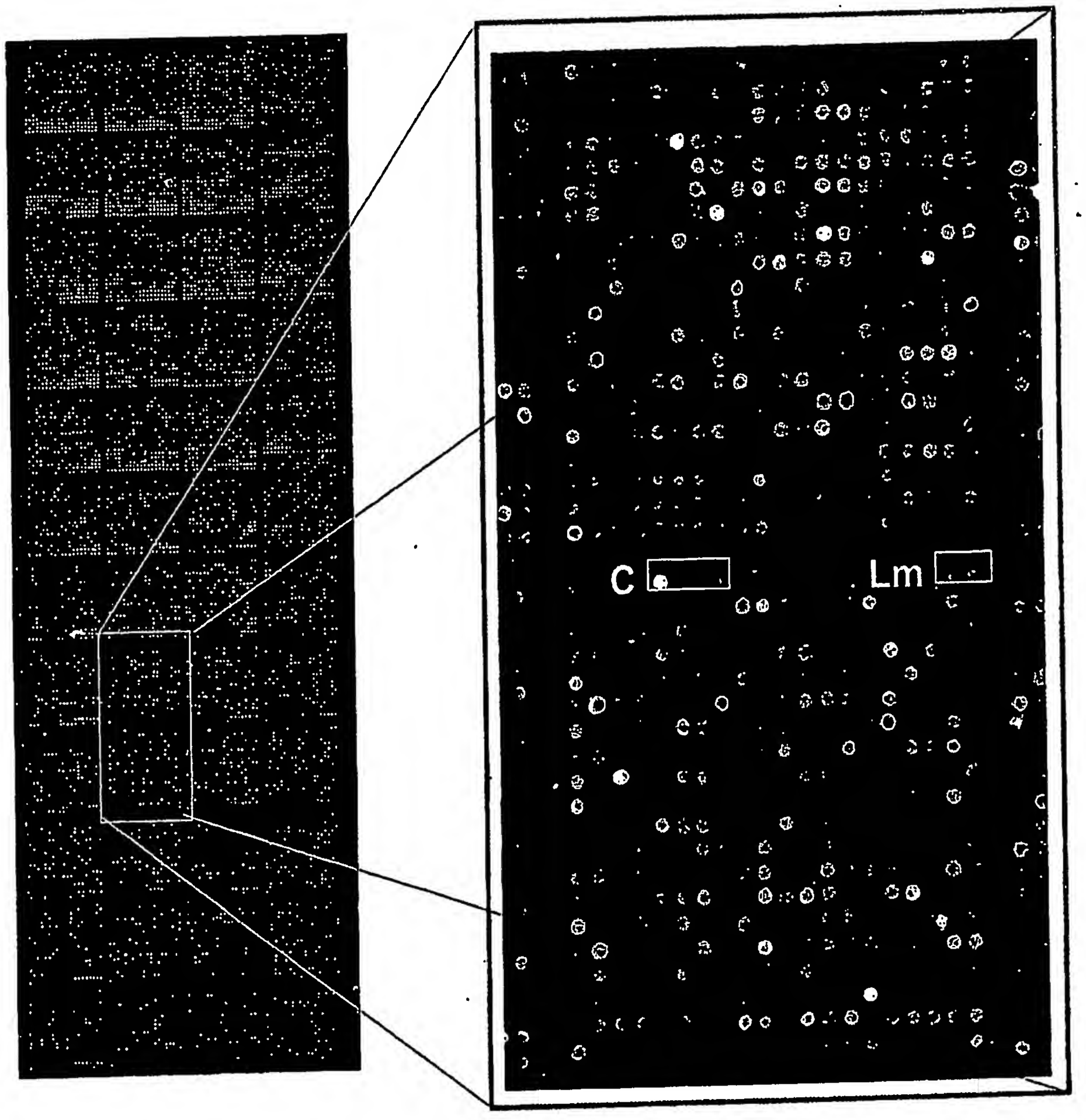
Fig. 8 NKI 18.5 K cDNA microarray



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CONCLUSIONS

81 GENE PROFILE FOR TAMOXIFEN REPONSE PREDICTION OF METASTATIC ER+ BREAST CANCER

PREDICTION OF PROGRESSIVE DISEASE (80%)

PREDICTION OF PROGRESSION-FREE SURVIVAL

GENE FUNCTION

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